Specificity tests: Response of habituated chaffinches (*Fringilla coelebs*) to modified forms of the species-specific alarm call pattern

Test	Stimulus	Duration of 'freezing' (mean and SD of the mean for $n = 4$, in % of the maximal value)
	e.s.: 10 trisyllabic 'pink'-calls with 1 sec intervals	81.5 ± 19.8
A	same as above, from another individual, more noisy	25.3 ± 19.5
B	e.s., half volume	0.0 ± 0.0
C	e.s., intervals 2 sec	4.3 ± 4.9
D	10 bisyllabic 'pink'-calls with 1 sec intervals	25.0 ± 21.6
E	e.s., double volume	9.0 ± 7.0
F	10 trisyllabic 'pink'-calls with 5 sec intervals	2.5 ± 1.5
G	15 bisyllabic 'pink'-calls with 1.3 sec intervals	12.3 ± 4.5
H	e.s., half speed	2.3 ± 2.3
I	e.s., double speed	33.3 ± 11.8
K	e.s. daily, alternating with the tests	0.0 ± 0.0

The first response to the experimental stimulus (e.s.) is given above for comparison.

mechanism. These criteria for a long-term habituation can be applied to our results: we could show that, under laboratory conditions, chaffinches habituate to a species-specific alarm call pattern. This type of behavioural adaption has also been observed in the acoustical control of bird flocks on airfields or in crops[5,6], but apparently has not been studied from a quantitative point of view nor in terms of learning processes. Habituation to visual stimuli, such as owl models and even living owls, has already been shown[7]. Phenomena of this type are probably basically different from short-term habituation which is also known for birds[8]. The almost complete loss of response to an enemy, as well as habituation to alarm calls, appears to have little adaptive value. In the field it would not occur to this extent, owing to habituation preventive factors such as the animal's continuously varying external and internal situation. Whether a generalized habituation to variant acoustical signals occurs, is to be tested by further experiments.

Zusammenfassung. Isoliert gehaltene Buchfinken (*Fringilla coelebs* L.) reduzieren fast alle ihre Verhaltensreaktionen auf Tonaufnahmen arteigener Alarmrufe bei täglich zweimaliger Darbietung desselben Musters nach ca. 6 Tagen auf einen Nullwert. Die fehlende Auslöserwirkung ist an das spezifische Muster der Gewöhnungsattrappe gebunden. Geringfügige Änderungen in verschiedenen Parametern riefen wieder Reaktionen hervor.

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The Growth-Retarding Effect of Guanidino-Methylated Arginines on the Tobacco Tissue Cultures

Guanidino-methylated derivatives of L-arginine/N^G-mono-methyl-L-arginine (MMA), N^G,N^G-dimethyl-L-arginine (DMA) and N^G,N'^G-dimethyl-L-arginine (DMA')/ have recently been detected in some animal[1] and plant proteins[2], as well as in free state in various tissues and biological fluids[3] as catabolic compounds of the enzymic methylated proteins.

The role of these methylated basic amino acids in the living organisms has not been cleared up so far. On the basis of the well-known growth-promoting effect of arginine[4], as well as of the tumour growth promoting[5] and growth promoting effect on the tobacco tissue cultures of N^ε-methylated lysines[6], we supposed the guanidino-methylated arginines to have an inhibitory effect on growth.

The present studies continued the comparative investigation of the effect of L-arginine and its 3 guanidino-methylated derivatives (MMA, DMA' and DMA) on the tobacco tissue cultures, and examined the stability of guanidino-methylated arginines in culture medium and in tissue cultures.

The test material used in our investigations was a secondary callus tissue isolated from tobacco (*Nicotiana tabacum* L.) stem. The tissue consisted of a yellowish-green cell population, was of intensive growth, and on standard culture medium did not show organ formation but only some tissue differentiation.

Of the amino acids used, L-arginine was a commercial product (Reanal Chemical Works, Budapest, Hungary), while the guanidino-methylated L-arginine derivatives were prepared by synthesis[7]. The different amino acids were applied at concentrations of 10.0–100.0 mg/l agar-agar culture medium[8]. In the Erlenmeyer dishes, each piece of tissue was placed on 50 ml culture medium, with an initial weight of 200 mg. The tissues grew for 62 days in a termostat of 28 ± 2°C temperature with a natural alternation of day and night.

The added amino acids can be extracted with 80% alcohol from tobacco tissue and culture medium after 62 days. The identification of amino acids in alcoholic extracts can be pointed out by one- and two-dimensional thin-layer chromatography on Fixion 50 × 8 chromato-

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Effect of L-arginine and its guanidino-methylated derivatives on the growth of tobacco tissue cultures

Treatment	End weight after 62 days (g)	Daily growth (mg)	Growth inhibition (%)
Control	16.38	264	0
A-10	16.22	261	0
A-100	8.49	137	49 ($P_{1\%}$)
MMA-10	9.89	159	40 ($P_{1\%}$)
MMA-100	2.32	37	87 ($P_{0.1\%}$)
DMA'-10	10.23	160	38 ($P_{1\%}$)
DMA'-100	3.84	62	77 ($P_{0.5\%}$)
DMA-10	10.62	170	36 ($P_{1\%}$)
DMA-100	3.10	44	79 ($P_{0.1\%}$)

Signs and abbreviations: A, L-arginine; MMA, N^G-monomethyl-L-arginine; DMA', N^G, N'^G-dimethyl-L-arginine; DMA, N^G, N^G-dimethyl-L-arginine; 10, 10 mg amino acid/l culture liquid; 100, 100 mg amino acid/l culture liquid.

plate containing Dowex 50×8 type ion exchange resin (Chinoin-Nagytétény, Budapest, Hungary), previously equilibrated with sodium citrate buffer (pH 3.28; 0.02 *N* Na^+). The eluting buffers used were various sodium citrate buffers[9].

As seen from the Table, at concentrations of 10 and 100 ppm MMA, DMA' and DMA respectively considerable growth inhibition could be attained after 62 days of culturing compared to the control.

After 62 days, the added L-arginine, and particularly its N^G-methylated derivatives, can be shown by ion exchange thin-layer chromatographic methods, even in alcoholic extracts of culture medium as in the tissue extracts. The 3 guanidino-methylated arginines show the following order of enrichment in the tissue compared to the control: control < arginine < MMA < DMA' < DMA. It is probable that the tobacco tissue cannot demethylate the 2 dimethyl-L-arginine. In the case of MMA the methyl group is less stable.

The data of our investigations prove that, in the case of treatment performed with guanidino-methylated arginines, a permanent inhibition of arginine incorporation, of vital importance for the tobacco tissue and its great stability, results in growth-retardion.

Zusammenfassung. Drei N^G-methylierte Derivate von L-Arginin hemmen das Wachstum der Tabak-Kallusgewebe in 10–100 ppm Konzentrationen.

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Responses of Cerebellar Units to Stimuli Simulating Sound Source Movement and Visual Moving Stimuli

In several publications the sensitivity of neuronal network in lobuli VI and VII of the cerebellum to visual and acoustic stimuli has been reported[1–4]. Neurones, mainly Purkyně cells, were found to be responsive to clicks, tones and flashes. Recently, responses of cerebellar neurones to moving visual stimuli were described[5]. In acoustically activated neurones, a high sensitivity to binaurally presented and time shifted clicks was demonstrated, whereas a lower responsiveness to tonal stimuli was found in comparison with neurones from specific auditory nuclei[6].

As is generally assumed, the cerebellum is involved in the control of body movements[7]; and it seems probable that information about the movement of acoustic and visual stimuli is transmitted to the cerebellum. We therefore attempted to explore the responses of lobulus VI and VII neurones to stimuli, which simulate the sound source movement[8,9], and simultaneously to investigate the reaction of the same neurones to moving visual stimuli.

Methods. Experiments were performed on cats immobilized by Diplacin (muscle relaxant, with effects

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